

WE CLAIM:

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1. A method for making a hypermutable cell, comprising the step of:  
introducing into a plant cell a polynucleotide comprising a dominant negative allele of a mismatch repair gene, whereby the cell becomes hypermutable.
  2. The method of claim 1 wherein the polynucleotide is introduced by transfection of a suspension of plant cells *in vitro*.
  3. The method of claim 1 wherein the mismatch repair gene is a plant *MutS* homolog.
  4. The method of claim 1 wherein the mismatch repair gene is a plant *MutL* homolog.
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  5. The method of claim 1 wherein the mismatch repair gene is a mammalian *PMS2*.
  6. The method of claim 1 wherein the mismatch repair gene is a mammalian *MLH1*.
  7. The method of claim 1 wherein the mismatch repair gene is a mammalian *PMS1*.
  8. The method of claim 1 wherein the mismatch repair gene is a mammalian *MSH2*.
  9. The method of claim 1 wherein the mismatch repair gene is an eukaryotic *mutS*.
  10. The method of claim 1 wherein the mismatch repair gene is an eukaryotic *mutL*.
  11. The method of claim 1 wherein the mismatch repair gene is a prokaryotic *mutS*.
  12. The method of claim 1 wherein the mismatch repair gene is a prokaryotic *mutL*.
  13. The method of claim 3 wherein the allele comprises a truncation mutation.
  14. The method of claim 4 where the allele comprises a truncation mutation.

~~15. The method of claim 5 where the allele comprises a truncation mutation.~~

16. The method of claim 15 wherein the allele comprises a truncation mutation at codon 134.

17. The method of claim 16 wherein the truncation mutation is a thymidine at nucleotide 424 of wild-type human *PMS2*.

18. The method of claim 1 wherein the polynucleotide is introduced into a plant cell in a plant to form a transgenic plant.

19. The method of claim 18 further comprising: growing the transgenic plant to form a mature transgenic plant.

20. The method of claim 19 wherein the mismatch repair gene is *PMS2*.

21. The method of claim 19 wherein the mismatch repair gene is a mammalian *PM82*.

22. The method of claim 19 wherein the mismatch repair gene is a mammalian *MLH1*.

23. The method of claim 19 wherein the mismatch repair gene is a mammalian *PMS1*.

24. The method of claim 19 wherein the mismatch repair gene is a mammalian *MSH2*.

25. The method of claim 19 wherein the mismatch repair gene is a plant *MutS* homolog.

26. The method of claim 19 wherein the mismatch repair gene is a plant *MutL* homolog.

27. The method of claim 19 wherein the mismatch repair gene is an eukaryotic *MutS* homolog.

28. The method of claim 19 wherein the mismatch repair gene is an eukaryotic *MutL* homolog.

29. The method of claim 19 wherein the mismatch repair gene is a prokaryotic *MutS* homolog.

30. The method of claim 19 wherein the mismatch repair gene is a prokaryotic *MutL* homolog.

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31. The method of claim 20 wherein the allele comprises a truncation mutation.
32. The method of claim 20 wherein the allele comprises a truncation mutation at codon 134.
33. The method of claim 20 wherein the truncation mutation is a thymidine at nucleotide 424 of wild-type *hPMS2*.
34. A homogeneous composition of cultured, hypermutable, plant cells which comprise a dominant negative allele of a mismatch repair gene.
35. The homogeneous composition of claim 34 wherein the mismatch repair gene is *PMS2*.
36. The homogeneous composition of claim 34 wherein the mismatch repair gene is mammalian *PMS2*.
37. The homogeneous composition of claim 34 wherein the mismatch repair gene is mammalian *MLH1*.
38. The homogeneous composition of claim 34 wherein the mismatch repair gene is mammalian *PMS1*.
39. The homogeneous composition of claim 34 wherein the mismatch repair gene is mammalian *MSH2*.
40. The homogeneous composition of claim 34 wherein the mismatch repair gene is a plant MutS homolog.
41. The homogeneous composition of claim 34 wherein the mismatch repair gene is a plant MutL homolog.
42. The homogeneous composition of claim 34 wherein the mismatch repair gene is an eukaryotic MutS homolog.
43. The homogeneous composition of claim 34 wherein the mismatch repair gene is an eukaryotic MutL homolog.
44. The homogeneous composition of claim 34 wherein the mismatch repair gene is a prokaryotic MutS homolog.
45. The homogeneous composition of claim 34 wherein the mismatch repair gene is a prokaryotic MutL homolog.

46. The homogeneous composition of claim 34 wherein the cells express a protein consisting of the first 133 amino acids of hPMS2.

47. A hypermutable transgenic plant wherein at least 50% of the cells of the plant comprise a dominant negative allele of a mismatch repair gene.

48. The hypermutable transgenic plant of claim 47 wherein the mismatch repair gene is a plant *MutS*.

49. The hypermutable transgenic plant of claim 47 wherein the mismatch repair gene is a plant *MutL*.

50. The hypermutable transgenic plant of claim 47 wherein the mismatch repair gene is a mammalian *MutS* homolog.

51. The hypermutable transgenic plant of claim 47 wherein the mismatch repair gene is a mammalian *MutL* homolog.

52. The hypermutable transgenic plant of claim 47 wherein the mismatch repair gene is an eukaryotic *MutS* homolog.

53. The hypermutable transgenic plant of claim 47 wherein the mismatch repair gene is an eukaryotic *MutL* homolog.

54. The hypermutable transgenic plant of claim 47 wherein the mismatch repair gene is a prokaryotic *MutS* homolog.

55. The hypermutable transgenic plant of claim 47 wherein the mismatch repair gene is a prokaryotic *MutL* homolog.

56. The hypermutable transgenic plant of claim 47 comprising a protein which consists of the first 133 amino acids of human *PMS2*.

57. A method for generating a mutation in a gene of interest in a plant cell, comprising the steps of:

growing a hypermutable plant cell comprising the gene of interest and a dominant negative allele of a mismatch repair gene;

testing the cell to determine whether the gene of interest harbors a mutation.

58. The method of claim 57 wherein the step of testing comprises analyzing a nucleotide sequence of the gene of interest.

59. The method of claim 57 wherein the step of testing comprises analyzing mRNA transcribed from the gene of interest.
60. The method of claim 57 wherein the step of testing comprises analyzing a protein encoded by the gene of interest.
61. The method of claim 57 wherein the step of testing comprises analyzing a phenotype caused by the gene of interest.
62. The method of claim 57 wherein the plant cell is made by the process of introducing a polynucleotide comprising a dominant negative allele of a mismatch repair gene into a plant cell, whereby the cell becomes hypermutable.
63. The method of claim 62 wherein the step of testing comprises analyzing a nucleotide sequence of the gene of interest.
64. The method of claim 62 wherein the step of testing comprises analyzing mRNA transcribed from the gene of interest.
65. The method of claim 62 wherein the step of testing comprises analyzing a protein encoded by the gene of interest.
66. The method of claim 62 wherein the step of testing comprises analyzing a phenotype caused by the gene of interest.
67. A method for generating a mutation in a gene of interest in a plant, comprising the steps of:
- growing a plant comprising the gene of interest and a polynucleotide encoding a dominant negative allele of a mismatch repair gene;
  - testing the plant to determine whether the gene of interest harbors a mutation.
68. The method of claim 67 wherein the step of testing comprises analyzing a nucleotide sequence of the gene of interest.
69. The method of claim 67 wherein the step of testing comprises analyzing mRNA transcribed from the gene of interest.
70. The method of claim 67 wherein the step of testing comprises analyzing a protein encoded by the gene of interest.

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71. The method of claim 67 wherein the step of testing comprises analyzing a phenotype caused by the gene of interest.

72. The method of claim 67 wherein the plant is made by the process of introducing a polynucleotide comprising a dominant negative allele of a mismatch repair gene into a plant, whereby the plant becomes hypermutable.

73. The method of claim 72 wherein the step of testing comprises analyzing a nucleotide sequence of the gene of interest.

74. The method of claim 72 wherein the step of testing comprises analyzing mRNA transcribed from the gene of interest.

75. The method of claim 72 wherein the step of testing comprises analyzing a protein encoded by the gene of interest.

76. The method of claim 72 wherein the step of testing comprises analyzing the phenotype of the gene of interest.

77. A hypermutable transgenic plant made by the method of claim 67.

78. The hypermutable transgenic plant of claim 77 wherein the mismatch repair gene is *PMS2*. B

79. The hypermutable transgenic plant of claim 77 wherein the mismatch repair gene is human *PMS2*.

80. The hypermutable transgenic plant of claim 77 wherein the mismatch repair gene is human *MLH1*.

81. The hypermutable transgenic plant of claim 77 wherein the mismatch repair gene is human *PMS1*.

82. The hypermutable transgenic plant of claim 77 wherein the mismatch repair gene is human *MSH2*.

83. The hypermutable transgenic plant of claim 77 wherein the allele comprises a truncation mutation.

84. The hypermutable transgenic plant of claim 77 wherein the allele comprises a truncation mutation at codon 134.

85. The hypermutable transgenic plant of claim 83 wherein the truncation mutation is a thymidine at nucleotide 424 of wild-type *PMS2*.

86. A method for generating a hypermutable plant, comprising the steps of:

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inhibiting endogenous mismatch repair (MMR) activity of a plant,  
whereby the plant becomes hypermutable.

87. The method of claim 86 wherein an endogenous plant MutS homolog is  
inhibited by mutagenizing an allele encoding the MutS homolog by  
introducing a mutation into said allele by homologous recombination.

88. The method of claim 86 wherein an endogenous plant MutL homolog is  
inhibited by mutagenizing an allele encoding the MutL homolog by introducing a  
mutation into said allele by homologous recombination.

89. The method of claim 86 wherein an endogenous plant MutL homolog is  
inhibited by introduction of a dominant negative allele of a plant MutL gene.

90. The method of claim 86 wherein an endogenous plant MutS homolog is  
inhibited by introduction of a dominant negative allele of a plant MutS gene.

91. The method of claim 86 wherein endogenous MMR activity is inhibited by  
introducing into said plant inhibitory peptides derived from plant MutS proteins.

92. The method of claim 86 wherein endogenous MMR activity is inhibited by  
introducing into said plant inhibitory peptides derived from plant MutL proteins.

93. The method of claim 86 wherein endogenous MMR activity is inhibited by  
introducing into said plant antisense *MutS* oligodeoxynucleotides.

94. The method of claim 86 wherein endogenous MMR activity is inhibited by  
introducing into said plant antisense *MutL* oligodeoxynucleotides.

95. The method of claim 86 wherein endogenous MMR activity is inhibited by  
introducing a polynucleotide encoding a MutS polypeptide from a lower organism into said plant  
and overexpressing in said plant the MutS polypeptide from the lower organism.

96. The method of claim 86 wherein wherein endogenous MMR activity is inhibited  
by introducing a polynucleotide encoding a MutL polypeptide from a lower organism into said  
plant and overexpressing in said plant the MutL polypeptide from the lower organism.

97. The method of claim 95 wherein the lower organism is a bacterium.

98. The method of claim 95 wherein the lower organism is a yeast.

99. The method of claim 95 wherein the lower organism is a unicellular organism.

100. The method of claim 96 wherein the lower organism is a bacterium.

101. The method of claim 96 wherein the lower organism is a yeast.

102. The method of claim 96 wherein the lower organism is a unicellular organism.

103. The method of claim 86 wherein endogenous MMR activity is inhibited by introducing a polynucleotide encoding a MutL polypeptide from a rodent into said plant and overexpressing in said plant the MutL polypeptide from the rodent.

104. The method of claim 86 wherein endogenous MMR activity is inhibited by introducing a polynucleotide encoding a MutS polypeptide from a rodent into said plant and overexpressing in said plant the MutS polypeptide from the rodent.

105. The method of claim 86 wherein endogenous MMR activity is inhibited by double stranded RNA interference of endogenous plant MMR.

106. A vector for introducing a dominant negative MMR allele into a plant, comprising: a dominant negative MMR allele under the transcriptional control of a promoter which is functional in a plant.

107. The vector of claim 106 wherein said vector further comprises an *Agrobacterium tumefaciens* T-DNA border repeat flanking the MMR allele.

108. The vector of claim 106 further comprising an origin of replication for independent replication in said plant.

109. The vector of claim 106 wherein the promoter is a Cauliflower Mosaic Virus promoter.

110. The vector of claim 106 wherein the promoter is a nopaline synthase promoter from *Agrobacterium tumefaciens*.

111. The vector of claim 106 further comprising a selectable marker.

112. The vector of claim 111 wherein the selectable marker is a neomycin phosphotransferase gene.

113. The vector of claim 106 wherein the MMR allele is PMS134.

114. The vector of claim 106 wherein the MMR allele is human PMS134.

115. The vector of claim 106 wherein the MMR allele is Arabidopsis PMS134.

116. An isolated and purified polynucleotide encoding Arabidopsis PMS2 as shown in SEQ ID NO: 14.

117. The isolated and purified polynucleotide of claim 116 comprising the sequence as shown in SEQ ID NO: 4.



118. An isolated and purified polynucleotide encoding Arabidopsis PMS134 as shown in SEQ ID NO: 16.

119. The isolated and purified polynucleotide of claim 118 comprising the sequence as shown in SEQ ID NO: 6.

120. An isolated and purified protein which is Arabidopsis PMS2 as shown in SEQ ID NO: 14.

121. An isolated and purified protein which is Arabidopsis PMS134 as shown in SEQ ID NO: 16.

122. A method for determining the presence of a mismatch repair (MMR) defect in a plant or a plant cell, comprising:

comparing at least two microsatellite markers in test cells or a test plant to the at least two microsatellite markers in cells of a normal plant;

identifying the test cells or test plant as having a mismatch repair defect if at least two microsatellite markers are found to be rearranged relative to the cells of the normal plant.

123. The method of claim 122 wherein a test plant is identified if at least one quarter of the markers compared are found to be rearranged.

124. The method of claim 122 wherein a test plant is identified if at least one third of the markers compared are found to be rearranged.

125. The method of claim 122 wherein a test plant is identified if at least one half of the markers compared are found to be rearranged.